bio-protocol Preprint

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Cell surface ELISA

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Updated date: Apr 13, 2020

An abbreviated version of this protocol was published in eLIFE in Jun 2019
A toolkit for studying cell surface shedding of diverse transmembrane receptors
DOI: 10.7554/eLife.46983

Detailed protocol

100 ng of Notch chimera constructs were transfected into U2OS cells in a sterile opaque tissue culture-treated 96-well plate (Corning 353296) in triplicate. 24 hr post-transfection, cells were washed once quickly with 100 uL PBS and fixed using 100 uL 4% PFA (Thermo Fisher 28906) for 20 min at room temperature, then washed quickly three times with 100 uL PBS. Cells were blocked in 100 uL TBS +5% milk at room temperature for 1 hr. Then, 50 uL Flag primary antibody (Sigma-Aldrich F1804) was added 1:250 in TBS +5% milk for 2 hr at room temperature. Cells were washed three times for 5 min each with 100 uL TBS +5% milk at room temperature. The cells were then incubated 50 uL 1:10,000 with an HRP secondary antibody for 1 hr at room temperature before being washed five times for 5 min each with TBS at room temperature. 100 uL chemiluminescent substrate (Dual-Luciferase Reporter Assay (Promega)) was added for 1 min before reading out on a luminescence plate reader.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Hayward, A. (2020). Cell surface ELISA. Bio-protocol Preprint. bio-protocol.org/prep272.
2. Hayward, A. N., Aird, E. J. and Gordon, W. R.(2019). A toolkit for studying cell surface shedding of diverse transmembrane receptors. eLIFE. DOI: 10.7554/eLife.46983

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